MICROBIOLOGY

Bacterial transport reversal

Some bacteria use multiprotein complexes to inject proteins into host cells. Components of these complexes have been linked to a nanotube-mediated route from host cells to bacteria that might provide food for disease-causing microbes.

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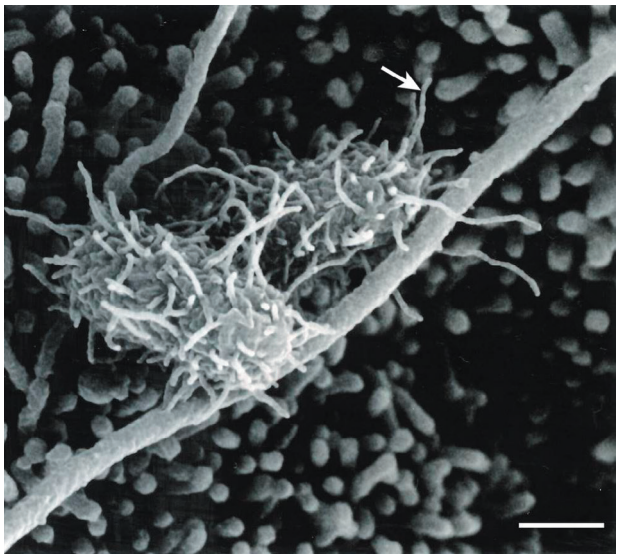
Among the most exciting developments of the past two decades of studies of the mechanisms by which bacteria cause disease was the discovery that many such microorganisms have the capacity to transfer bacterially encoded proteins directly into the cells that they infect[1]. The transferred proteins are known as effectors, and they fulfil diverse roles in modulating cellular processes to promote bacterial infection. This remarkable feat of transfer is achieved by protein complexes that form injection machines. One of the most widespread injection machines is the type III secretion system (T3SS), which functions in many disease-causing bacteria[2]. Writing in *Cell*, Pal *et al.*[3] report the intriguing finding that a subset of the components that make up the T3SS in a disease-causing strain of the bacterium *Escherichia coli* are repurposed to aid the generation of a nanotube-like structure on the bacterial cell surface that might be involved in transporting molecules in the opposite direction: from host cell to bacterium.



C. C. GINOCCHIO *ET AL./CELL*

Figure 1 | Bacterial nanotubes. Bacterial nanotubes (arrow) can form connections between mammalian host cells and bacterial cells (shown here are nanotubes on the surface of *Salmonella* Typhimurium bacteria that are in contact with canine kidney cells grown *in vitro*)[4]. Pal *et al.*[3] provide evidence to suggest that such connections can be used by bacteria to gain nutrients from mammalian cells. Scale bar, 0.5 micrometres.

The origins of this discovery can be traced back to previous studies[4,5], which documented the presence of nanotube structures on the surface of some species of bacterium. Although the composition of the nanotube structures is not fully understood, it is known that they can form bridges between neighbouring bacterial cells[6], or connections between bacteria and mammalian host cells during infection (Fig. 1)[4]. The function of these structures has remained elusive, although it has been suggested that they are involved in transporting molecules between bacteria[6] or facilitating the propagation of signals from bacteria to mammalian cells[4].

Pal *et al.* present data that implicate nanotube structures in the potential direct scavenging of nutrients from host cells. The authors engineered *E. coli* to express a fluorescent protein only when the bacterial cells contained normal levels of the amino acid proline. If the authors grew the cells under conditions of amino-acid starvation, the fluorescent protein was not expressed. But if bacteria under such conditions were also in contact with mammalian cells, they expressed the fluorescent protein. This indicates that the microbes responded as though they were acquiring nutrients.

These experiments, however, could not disentangle whether the nanotubes are used to forage nutrients directly, and, if they are, whether they transport nutrients from the host-cell surface or from the cytoplasm of the host cell's interior. The latter scenario would presumably require nanotubes to have the capacity to pierce the cell membrane of the host cell. The authors also report that a membrane-permeable dye can be transferred from a mammalian host cell grown *in vitro* to a bacterium only when both types of cell are in close contact.

However, there is no direct evidence that the nanotubes do, in fact, mediate molecular transport — the authors' data provide only a correlation between the presence of these structures and the nutritional response of the bacteria or the acquisition of the dye. Alternative explanations for the observations have therefore not been ruled out, including the involvement of nanotubes in facilitating intimate interactions between bacteria and host cells that lead to nutrient acquisition through another mechanism. Moreover, the identity of the molecule or molecules that usually travel by the authors' proposed route remains unknown. Nevertheless, although questions remain, the data are compelling enough to support Pal and colleagues' model.

Experiments by Pal *et al.* indicate that nanotube formation depends on the expression of only a subset of the components that form the T3SS in *E. coli*. Also known as the injectisome, the T3SS is composed of two major multiprotein substructures: a protein complex called the cytoplasmic sorting platform, which is responsible for the selection of effectors to be delivered by the T3SS; and the needle complex, which mediates the passage of effectors across the bacterial cell membrane. Deep within the needle complex resides the export apparatus — a group of several membrane proteins that aid the passage of effectors through the inner membrane of the bacterial cell (some bacterial cells are surrounded by both inner and outer membranes). These export-apparatus proteins make up the subset of T3SS components that are needed to drive nanotube formation in the authors' experimental system.

Pal and colleagues found that expression of the export apparatus alone is sufficient for nanotubes to form in *E. coli*. This observation hints at the mechanisms that might lead to nanotube assembly: given that the export-apparatus proteins reside in the inner membrane of the bacterium, could they somehow stimulate the membrane to form tubules, leading to nanotube generation? The proteins of the export apparatus are evolutionarily highly conserved, and the authors report that nanotubes could form in *E. coli* that were engineered to express the export apparatuses of other bacterial species' T3SSs. Nanotubes were also made when the authors engineered *E. coli* to express components of a bacterial structure called the flagellum, which has a role in

microbial motility and contains proteins that are related to those that form the T3SS.

Given the location of the export apparatus at the core of the T3SS, the use of export-apparatus proteins to drive nanotube formation would be incompatible with these components also functioning as part of an injectisome. This suggests that a regulatory mechanism would be needed to ensure that export-apparatus proteins are assigned to form either an injectisome or a nanotube. Intriguingly, in the T3SSs of most species of bacterium, the genes that encode the export apparatus are clustered together in a different genetic region from that containing the genes that encode other components of the needle complex. This organization could aid the differentially regulated production of the needle complex and the export apparatus.

However, Pal *et al*. present some indirect evidence that individual bacterial cells could be simultaneously engaged in nutrient foraging using nanotubes and effector injection through the injectisome. This would suggest a more-complex regulatory mechanism for the system than just differential gene expression of the components. Nanotubes have been found on the surfaces of bacterial cells that do not seem to be engaged in the T3SS-mediated injection of effectors[4]. It is therefore possible that, before making contact with host cells, certain populations of bacterial cells are poised either to assemble injectisomes or to form nanotubes.

Pal and colleagues' study raises many questions that are worthy of further research. How are the nanotubes assembled? Does the transport occur in only one direction — for example, from the host cell to the bacterium — or can it be bidirectional? Is the transport selective for certain types of compound? Stay tuned for the answers because, undoubtedly, more surprises are yet to come. ■

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LINGUISTICS

The origin and spread of Sino-Tibetan languages

A robust computational approach with added finesse provides evidence to support the view that the Sino-Tibetan languages arose in northern China and began to split into branches about 5,900 years ago. SEE LETTER P.112

RANDY J. LAPOLLA

The location and timing of the emergence of the Sino-Tibetan language family has long been debated. This family has around 1.5 billion speakers worldwide, the second largest number of speakers globally after those who speak languages in the Indo-European family. One school of thought is that the ancestral language (Proto-Sino-Tibetan) from which all the Sino-Tibetan languages evolved originated in northern China around 4,000–6,000 years ago[1,2]. An alternative view is that it arose 9,000 years ago in southwest China or northeast India[3,4].

Zhang *et al*.[5] report a study on page 112 that might settle this debate. The authors gathered evidence about the Sino-Tibetan language family and its speakers from disciplines including genetics, computational biology, linguistics, archaeology and anthropology, and also compiled information about the development of agriculture and its possible effects on human migrations in the region. They then used a method of probability testing to assess the different language family trees that could be made on the basis of this evidence.

Historical linguists seek to determine the relationships between languages, and usually take an approach called the comparative method. They look for cognate words in different languages — words that have similar meanings and that can be shown to have a shared origin in a word from an earlier, ancestral language. Linguists then try to explain why the words often don't look exactly alike: the changes that the sounds went through, what additions were made to the words, and what led to the words being used, in some cases, for different meanings in related languages. For example, work in Indo-European linguistics has determined that the English word cow and the French word *boeuf* are part of a family of cognate words that have descended from a reconstructed Proto-Indo-European root word, *gwou- (the asterisk indicates a reconstructed form and the hyphen that it is a root that formed a number of different words)[6]. Understanding such changes enables language families such as the Indo-European family to be split into branches, such as the Romance, Germanic and Slavic languages, on the basis of shared changes.

The use of particular words found to be cognate, together with evidence from other fields, can help inferences to be made about the relationship of languages to human migrations,

Figure 1 | Site of origin of the Sino-Tibetan languages. Zhang *et al*.[5] present the results of a probability-testing approach used to analyse data relating to the origins and spread of the Sino-Tibetan languages, which are spoken today by 1.5 billion people. Their analysis indicates that, consistent with one current model[1], the ancestral form of the language originated approximately 5,900 years ago in northern China, in the basin of the Yellow River. They identify the origin and earliest spread of the languages as being associated, respectively, with the Yangshao culture and the later Majiayao[7] (cultures indicated in shaded regions).